

## COMPOSITION OF CARTILAGE THERAPEUTIC AGENTS AND ITS APPLICATION

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

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The present invention relates to a composition of cartilage therapeutic agents for clinical transplantation into articulatio genu (knee joints) or ankle joints, and its application. In particular, it has clinical significance for symptomatic cartilage defect regions of the femoral condyle (medial, lateral, or trochlear) and bone cartilage defect regions of the talus (anklebone),  
10 in human or animal hosts.

#### 2. Related Prior Art

Generally, cartilage tissues constituting vertebral joints, once damaged, cannot be  
15 regenerated to their original state in vivo.

Where articular cartilage tissues are damaged, normal daily activity is limited by severe pain. Initial articular cartilage tissue damage progresses to a chronic state, called fatal degenerative arthritis, which interferes with normal life.

Meanwhile, in order to treat articular cartilage damage, presently, one method of treatment  
20 is to grind the damaged articular surface, thus rendering it soft; another method is to induce micro fracture or make a small hole at the articular surface.

However, such methods have disadvantages such as recurrence of symptoms within several weeks thus making it impossible to return to a normal lifestyle. In addition, fibrous

tissues prepared via such treatment methods are qualitatively completely different from normal cartilage tissues.

Further, where the articular cartilage tissues are significantly damaged, regeneration of cartilage tissues becomes impossible. Therefore, when cartilage tissues are incapable of being regenerated due to a severely damaged state thereof, given conventional treatment methods, the damaged articular cartilage must be completely removed and replaced with a metal or plastic artificial joint.

Up to now, such an artificial joint replacement technique is known as the only method for treating cartilage damage that is too severe to be treated using regeneration techniques.

10 In the US alone, 300,000 of the above-mentioned artificial knee joint replacements are conducted per year.

However, the artificial articular cartilage is incapable of permanent use, because the device has a service life of only about 10 years. Additionally, the price of the device is more than 4,000 dollars, thus imposing a heavy economic burden on patients.

15 Further, the frequency of complication recurrence for such artificial articular cartilage is excessively high, therefore it is inappropriate for active young people.

Recently, in order to treat damaged articular cartilage, a great deal of attention has been directed to autologous chondrocyte transplantation as a new treatment method based on tissue engineering.

20 This technique was first investigated in the Hospital for Joint Disease, in New York, USA, and further developed by the University of Gothenburg and Sahlgrenska University Hospital in Sweden.

Autologous chondrocyte transplantation is a method for treating damaged cartilage in a

patient's joint, involving collecting cartilage from a lesser-weight bearing area of the patient suffering from damaged cartilage of knee joints followed by separation of chondrocytes, culturing the separated chondrocytes to obtain an amount of chondrocytes sufficient for treating cartilage defect regions, and then transplanting the cultured chondrocytes into cartilage defect regions of the patient to restore damaged articular cartilage.

Performance of this autologous chondrocyte transplantation was verified as a fundamental joint treatment based on various clinical results, but necessarily involves invasive procedures including collecting a sample from the periosteum and, when transplanting into an affected part, injecting the chondrocyte therapeutic agent.

In addition, the autologous chondrocyte transplantation has various disadvantages such as a large incision of the knee associated with periosteum sample collection, leakage possibility of the chondrocyte therapeutic agent due to incomplete suturing between the surgical site and the periosteum, excessive time consumption in suturing between the periosteum and articular defect region, and the like.

## SUMMARY OF THE INVENTION

In order to solve the aforementioned problems, an objective of the present invention is to provide a cartilage therapeutic composition capable of avoiding difficulties due to periosteum sample collection, suturing, incomplete suturing between the periosteum and transplanted site, large incisions, and the time required for surgery, upon transplanting the cartilage therapeutic agent, and a method of using the same.

Another objective of the present invention is to provide a cartilage therapeutic

composition providing minimum loss of cartilage therapeutic agent, more complete cartilage regeneration effects, reduction of surgical operation time and utilization of an arthroscope, and a method of using the same.

In accordance with an aspect of the present invention, the above and other objects can be  
5 accomplished by the provision of a cartilage therapeutic composition comprising a mixture of components of chondrocytes isolated and expanded or differentiated from a host, thrombin and a fibrinogen matrix.

In accordance with another aspect of the present invention, there is provided a method of  
10 using a cartilage therapeutic composition, comprising: preparing components of chondrocytes isolated and expanded or differentiated from a host; preparing thrombin; preparing a fibrinogen matrix; treating a cartilage defect region; and mixing the chondrocyte components, thrombin and fibrinogen matrix and injecting the resulting mixture into the cartilage defect region.

#### BRIEF DESCRIPTION OF THE DRAWINGS

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Fig. 1 is a graph showing the strength of a matrix for transplanting chondrocytes in accordance with the present invention, with respect to an injection concentration of fibrinogen and thrombin.

Fig. 2 is a photograph of the matrix of Fig. 1 when fibrinogen was injected in an amount  
20 of 200 mg/mL (A), 160 mg/mL (B) and 20 mg/mL (C), respectively.

Fig. 3 is a photograph showing the trimmed cartilage defect region in which a matrix in accordance with the present invention was transplanted.

Fig. 4 is a photograph showing a transplanted site in which a matrix in accordance with

the present invention is transplanted.

Fig. 5 is a photograph showing an injection state of a cartilage therapeutic composition after periosteum suture in accordance with another example of the present invention.

Fig. 6 is a photograph showing an injection state of a cartilage therapeutic composition 5 after previously forming a connection hole at a cartilage defect region, in accordance with another example of the present invention.

Fig. 7 is a photograph showing chondrocyte differentiation patterns in a fibrinogen matrix in accordance with the present invention.

Fig. 8 is a photograph showing chondrocyte differentiation patterns in a fibrinogen matrix 10 containing 10% collagen and 10% hyaluronic acid in accordance with another example of the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

15       Hereinafter, the composition of cartilage therapeutic agents of the present invention will be described in more detail.

      The present invention may improve, maintain and restore the function of tissues by using tissue engineering involving replacement of tissues and organs.

20       In accordance with the present invention using tissue engineering, provided is a cartilage therapeutic composition formed by mixing components of chondrocytes isolated and expanded or differentiated from a host such as human or animal, and thrombin and a fibrinogen matrix.

      In addition, the present invention requires a matrix in which specific cells and growth factors may be embedded as a tissue replacement, and uses in vivo components as a matrix, such

as constituents of extracellular matrix (ECM), and a natural polymer as a matrix replacement.

The interaction between the chondrocyte and matrix directly promotes cell adhesion, migration, growth, differentiation and apoptosis.

Further, such interaction regulates the activity of cytokines and growth factors, and activates an intracellular signal transduction process resulting in rapid growth and restoration of tissues of interest.

Meanwhile, a method of using a cartilage therapeutic composition in accordance with the present invention involves preparing components of chondrocytes isolated and expanded or differentiated from humans or animals, and preparing thrombin that is used as a clotting factor.

10 This is followed by preparation of a fibrinogen matrix that is to be mixed with the chondrocyte components and thrombin, that is, components such as fibrinogen, collagen, hyaluronic acid, glycosaminoglycan (hereinafter, "GAG") and aprotinin.

The fibrinogen matrix must contain fibrinogen. Fibrinogen may be used alone in an appropriate concentration, or the fibrinogen matrix may be formed by mixing fibrinogen and 15 other matrix components such as collagen, hyaluronic acid, GAG and aprotinin in a suitable ratio.

20 The aprotinin serves to inhibit degradation of fibrinogen by plasmin, thus enabling the shape of the cartilage therapeutic agent (a mixture of thrombin, chondrocyte components and fibrinogen) to be maintained in a predetermined form for a longer period of time, when components of the cartilage therapeutic agent are mixed and solidified.

At this time, the cartilage defect region, into which the cartilage therapeutic agent will be transplanted, is first trimmed, and then a hole having a diameter of 2 to 3 mm and a depth of 3 to 5 mm is made inside a transplantation site for transplanting, followed by spraying thrombin.

Then, the cartilage therapeutic agent composed of a mixture of chondrocyte components, thrombin and a fibrinogen matrix is injected into the cartilage defect region to which thrombin was sprayed.

Subsequently, a high concentration of thrombin is sprayed for engrafting and shape 5 maintenance of the cartilage therapeutic agent onto the upper surface of the cartilage defect region into which the cartilage therapeutic agent was injected.

In the present invention, the chondrocyte components are prepared and used by separating chondrocytes from normal cartilage tissue using a suitable enzyme, and incubating the separated cells in a culture medium to obtain a culture containing more than 10<sup>6</sup> cells/mL.

10 In addition, the fibrinogen is restored to its original state from lyophilized fibrinogen using conventional cell culture media such as DMEM (Dulbecco's Modified Eagle's Medium), Ham's F-12 medium, DMEM/F-12, IMDM (Iscove's Modified Dulbecco's Medium), McCoy's 5A Medium, MEM (Minimum Essential Medium),  $\alpha$ -MEM, RPMI 1640 medium, Leibovitz's L-15 Medium, and Eagle's basal Medium.

15 The thrombin is also restored to its original state from lyophilized thrombin using the above-mentioned culture media including DMEM medium.

Further, the fibrinogen matrix is formed by mixing the fibrinogen with at least one of collagen, hyaluronic acid and GAG, or aprotinin in a predetermined concentration.

20 In this connection, fibrinogen is a substance present in the blood, which plays a role in clotting as it reacts with thrombin and then is converted into fibrin that is then clotted by CaCl<sub>2</sub> and a factor XIII added to thrombin, thus achieving a hemostatic function.

In addition, the culture medium for lysing the lyophilized fibrinogen may contain less than 10% of FBS (fetal bovine serum), FCS (fetal calf serum), calf serum, horse serum or human

serum and in order to prevent infection with microorganisms, antibiotics such as penicillin G, kanamycin, gentamycin and streptomycin or antifungal agents such as amphotericin B and nystatin may be added thereto in an appropriate amount.

5 Additionally, as the culture medium for lysing the lyophilized fibrinogen, all the conventional media for cell culture may be used including McCoys 5A medium, Eagle's basal medium, Glasgow minimum essential medium, Ham's F-12 medium, Iscove's modified Dulbecco's medium, Liebovitz' L-15 medium and RPMI 1640 medium.

10 In a specific embodiment of the fibrinogen matrix, the fibrinogen is used alone in concentrations between 20 mg/mL and 200 mg/mL, depending on the conditions of the transplantation site and patient.

When present at a high concentration, the fibrinogen is relatively hard and forms a dense matrix, but leads to increased degradation time resulting in sensation of foreign matter in the body after use thereof.

15 In contrast, when it is present at a low concentration, degradation of the fibrinogen within tissue rapidly progresses, but a loose matrix is formed, thus resulting in difficulty of replacement with cartilage.

20 Further, the fibrinogen matrix may contain 0.01 mg/mL to 20 mg/mL of collagen, 0.1 mg/mL to 20 mg/mL of hyaluronic acid and 0.1 mg/mL to 20 mg/mL of GAG (glycosaminoglycan), and 1 to 3000 KIU/mL of aprotinin alone or in any combination thereof, in addition to 20 mg/mL to 200 mg/mL of fibrinogen.

The above-mentioned collagen, hyaluronic acid, GAG, and aprotinin are substances present in host animals, and when they are administered in a predetermined concentration, the effects on cells do not significantly vary but hardening time increases, leading to retardation of

surgical operation time.

Additionally, aprotinin is a substance that inhibits degradation of fibrin in vivo and thereby assists in a hemostatic function, and when the added amount of the aprotinin increases, the degradation rate of fibrin decreases. Therefore, it is possible to control the degradation rate of the matrix by adjusting the aprotinin concentration.

Generally, cartilage of vertebral animals consists of chondrocytes and a matrix. The matrix is largely composed of GAG containing collagen and hyaluronic acid and has no blood vessels but rather receives nutrients from synovial fluid.

The main component of synovial fluid is hyaluronic acid, and therefore, the use of a mixture containing such a component provides excellent cartilage regeneration capabilities.

0.01 IU/mL to 50 IU/mL of the prepared thrombin is mixed with the cartilage therapeutic agent of the present invention and therefore, when mixing cartilage components with the fibrinogen matrix, the thrombin converts fibrinogen into fibrin.

Therefore, by adjusting the concentration of the thrombin added to the mixture of the cartilage therapeutic agent, it is possible to control the hardening rate of the cartilage therapeutic agent, and also to control the physical properties of the fibrinogen matrix.

Where the concentration of thrombin is low, the matrix is composed of thick fibrous materials and has a large pore size. Therefore, it is advantageous to incorporate cells and physiologically active materials, but this leads to retardation of hardening time thus impeding the achievement of the desired objects.

Conversely, where the concentration of the thrombin is high, the matrix is composed of thin fibrous materials and has a small pore size. Therefore, it is advantageous to allow for easy ingrowth while blocking cell penetration, but this leads to rapid hardening time resulting in

extremely low workability due to hardening of the matrix during mixing of respective components.

Therefore, the thrombin concentration is preferably within the range of 0.01 IU/mL to 50 IU/mL, and the injected thrombin hardens within 1 to 10 min. In addition, preferably, when the 5 thrombin is mixed with the matrix of the present invention, the thrombin is used at a low concentration, and when it is used before or after injection of the composition, the thrombin is used at a high concentration.

As a result, it is possible to obtain a matrix having mechanical strength and flexibility suitable for regeneration of cartilage tissues.

10 It is also possible to easily modify the shape of the matrix such that it is adapted to the various possible configurations present in the damaged cartilage region, thus enhancing the convenience of the associated surgical operation, and also allowing the matrix to be continuously positioned in a damaged region while maintaining a predetermined shape thereof after transplantation, leading to promotion of in vivo expansion and differentiation of the grafted 15 chondrocytes.

In the cartilage defect region into which a composition for transplanting chondrocytes according to the present invention is to be transplanted, the defect region is first trimmed, zero to five holes having a diameter of 2 to 3 mm and depth of about 3 to 5 mm are made inside the transplanting site depending on the size of the affected part, followed by stanching, and then 20 100 IU/mL to 1000 IU/mL of high concentration thrombin is sprayed onto the surface of the affected part to increase adhesion of the cartilage therapeutic agent.

At this time, the thrombin serves to induce close binding between the cartilage therapeutic agent and the cartilage defect region into which the cartilage therapeutic agent is to be

transplanted, thus increasing cartilage regeneration density while withstanding external impact.

In addition, a cartilage therapeutic composition is injected into a cartilage defect region of a human or animal patient.

Injection of the cartilage therapeutic composition is performed by slow injection to the 5 perforated part of the cartilage defect region followed by injection to fill all the remaining defect parts.

Since the time required for conversion of fibrinogen into fibrin following mixing between fibrinogen and thrombin is concentration-dependent, the cartilage therapeutic agent is injected to the defect region within a suitable period of time between about 1 and 10 min.

10 Further, when transplanting the cartilage therapeutic composition into the cartilage defect region, 100 IU/mL to 1000 IU/mL of high concentration thrombin is sprayed onto the transplanted cartilage therapeutic composition composed of chondrocyte components, thrombin and fibrinogen, in order to assist in engraftment and shape maintenance thereof, thereby forming a protective film to protect the cartilage therapeutic agent during cartilage regeneration.

15 The transplanted site is left for about 5 minutes so that it hardens completely during conversion of fibrinogen into fibrin by the action of thrombin.

Now, the present invention will be described in more detail with reference to the following Examples. These examples are provided only for illustrating the present invention and should not be construed as limiting the scope and spirit of the present invention.

20

## EXAMPLES

### Experimental Example 1

In order to determine appropriate physicochemical properties of various matrices used in

transplanting a cartilage therapeutic agent, commercially available lyophilized thrombin was lysed to a concentration of 2 IU/ml to 10 IU/mL.

2 IU/ml to 10 IU/mL of thrombin was added to convert fibrinogen, a main matrix, into fibrin.

5        Additionally, collagen, hyaluronic acid and GAG were added at 0.01 mg/mL to 20 mg/mL, 0.1 mg/mL to 20 mg/mL and 0.1 mg/mL to 20 mg/mL, respectively, to prepare the respective matrix.

Each matrix thus prepared was mixed and the resulting hardness, strength and bending strength thereof were measured.

10      First, 1 mL of fibrinogen having a concentration of 20 mg/mL to 200 mg/mL and 1 mL of thrombin having a concentration of 2 IU/mL to 10 IU/mL were mixed and the hardness and strength of the resulting respective composition were measured.

15      In addition, the practical cartilage therapeutic agent and the matrix having the above-mentioned concentration were mixed and then the strength of the resulting composition was measured.

As a result, as shown in Fig. 1, through various clinical trials, when transplanting a cartilage therapeutic composition consisting of a chondrocyte component-thrombin-fibrinogen mixture, a suitable strength thereof was about 160 to 170 g/cm<sup>2</sup>. Based on the results measured as described above, strength of the composition was obtained as follows: 166 g/cm<sup>2</sup> for 2 IU/mL of thrombin and 20 mg/mL of fibrinogen; 170 g/cm<sup>2</sup> and 165 g/cm<sup>2</sup> for 4 IU/mL of thrombin and 160 mg/mL or 200 mg/mL of fibrinogen, respectively; 170 g/cm<sup>2</sup> and 166 g/cm<sup>2</sup> for 8 IU/mL of thrombin and 160 mg/mL or 200 mg/mL of fibrinogen, respectively; and 171 g/cm<sup>2</sup> and 169 g/cm<sup>2</sup> for 10 IU/mL of thrombin, and 160 mg/mL or 200 mg/mL of fibrinogen,

respectively. Consequently, suitable concentrations of fibrinogen and thrombin for transplantation and characteristics as shown in Fig. 2 were obtained.

Differences between physical properties with respect to the fibrinogen concentrations of the composition showed the characteristics shown in the photographs of Fig. 2 (A: 200 mg/mL, 5 B: 160 mg/mL, and C: 20 mg/mL from left to right).

When measuring hardness of each composition in which each matrix (i.e., collagen, hyaluronic acid or GAG) was mixed with appropriate concentrations of fibrinogen and thrombin, the measured strength of the cartilage therapeutic composition was about 154 to 159 g/cm<sup>2</sup>, with a decrease of about 1 to 6 g/cm<sup>2</sup> in strength, as compared to the above-measured results.

10 This decrease in strength may be controlled by adjusting the concentrations of matrix components such as collagen, hyaluronic acid and GAG, or using any combination thereof.

In addition, since the composition of the matrix varies depending on conditions and characteristics of the patient, the above-mentioned range of fibrinogen and thrombin concentrations is used in combination with matrix components such as collagen, hyaluronic acid 15 and GAG.

Further, cartilage generation effects can be obtained by addition of collagen or hyaluronic acid in the case of elderly patients, and by use of composition in combination with GAG or hyaluronic acid in the case of young patients suffering from severe trauma, e.g. due to a traffic accident.

## 20 Experimental Example 2

Polymerization time required imparting the injected fibrinogen and thrombin with strength of 160 to 170 g/cm<sup>2</sup>, suitable for use as a cartilage therapeutic agent, is shown in Table 1.

Table 1

Fibrinogen Conc. (mg/mL)	Thrombin Conc. (IU/mL)	2	4	8	10
20		225	213	160	118
40		246	236	181	126
80		281	275	208	179
120		312	299	231	199
160		359	325	269	216
200		379	367	301	290

That is, a low concentration of thrombin exhibited a slow polymerization time and formed a thin fibrous structure having a pore size of more than about 5  $\mu\text{m}$ , while a high concentration of thrombin exhibited a rapid polymerization time and formed a structure having a small pore size of less than about 1  $\mu\text{m}$ .

Additionally, it can be seen that the composition composed of thrombin and fibrinogen took 2 to 7 minutes to exhibit strength of 150 to 180 g/cm<sup>2</sup>, suitable for use in a cartilage therapeutic agent.

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#### Example 1

##### Injection of cartilage therapeutic composition by arthroscope:

First, cartilage tissue was collected from an animal or human host and chondrocytes were isolated from the tissue. Then, the isolated cells were cultured to prepare a cartilage therapeutic agent consisting of 0.3 to 0.5 mL of DMEM and 9x10<sup>6</sup> to 1.5x10<sup>7</sup> chondrocytes.

Then, as a commercially available medical product, a fibrin sealant was prepared and a DMEM was added to a vial containing lyophilized fibrinogen such that fibrinogen was lysed to a concentration of 100 mg/mL to 200 mg/mL.

Additionally, lyophilized thrombin was lysed to a concentration of 500 IU/mL and then the resulting thrombin solution was added to the cartilage therapeutic agent in the concentration of 1 IU/mL to 10 IU/mL.

After completion of the above-mentioned process, a cartilage defect region in the patient's 5 knee was readied for transplantation by trimming the cartilage defect region using the arthroscope.

Next, the damaged cartilage region of the patient was cleanly trimmed using surgical instruments and then three to five holes having a diameter of 3 mm and depth of 5 mm were made within the damaged region using a surgical drill, depending on the size of the defect area.

10 Then, debris of cartilage and bone was removed from the defect region and stanchied to finish the necessary steps before transplantation.

After completion of these steps, chondrocyte components, thrombin and a matrix containing at least fibrinogen were mixed to prepare a cartilage therapeutic agent.

15 In this connection, since fibrinogen reacts with thrombin followed by conversion into fibrin which in turn clots, the cartilage therapeutic agent should be transplanted within 5 minutes into a transplantation site.

Subsequently, 500 IU/mL of the previously prepared thrombin solution was sprayed at the transplantation site of the cartilage therapeutic composition using a spray tip and then was gently wiped with gauze.

20 Then, the cartilage therapeutic composition, composed of a mixture of chondrocyte components, thrombin and fibrinogen, was slowly injected using a syringe through the previously perforated holes in the affected part into the entire area sequentially.

Injection of the composition should be rapidly performed without formation of bubbles,

and immediately after completion of the injection, 500 IU/mL of high concentration thrombin solution was evenly sprayed using the spray tip.

#### Example 2

5      Transplantation of cartilage therapeutic agent in which collagen was added to a composition of chondrocyte components, thrombin and fibrinogen mixture, under arthroscope:

As described above, chondrocyte components, thrombin and fibrinogen were prepared.

In order to reinforce elasticity and to obtain cartilage regeneration effects in a patient exhibiting decreased articular elasticity, a fibrinogen matrix mixed with collagen may be used for  
10     transplantation.

The fibrinogen matrix is made up of fibrinogen with collagen, hyaluronic acid and GAG added thereto. Upon combined use of fibrinogen and collagen, for a 50-year old male patient, a tenth or a fifth concentration of collagen may be used alone or in an admixture, relative to the fibrinogen matrix concentration, even though collagen concentration varies depending on  
15     conditions of the patient.

For example, in the case of the matrix for transplantation into a 52-year old male patient, fibrinogen and collagen were prepared at concentrations between 80 mg/mL to 160 mg/mL and 10 mg/mL to 20 mg/mL, respectively. Then, 0.3 mL of fibrinogen and 0.1 mL of collagen were mixed to prepare a fibrinogen matrix for transplantation.

20     Next, a hole for inserting an arthroscope was made at an affected part, as in Example 1, and the arthroscope was then inserted there through to cleanly trim the transplantation site. Then, transplantation was readied by making about 4 holes having a diameter of 3 mm and depth of 5 mm at the affected part (lesion size of patient is about 4 cm<sup>2</sup>).

Subsequently, 1 IU/mL to 10 IU/mL of thrombin was added to chondrocyte components, and then the cartilage therapeutic composition, in which the fibrinogen matrix containing a mixture of chondrocyte components and collagen was mixed, was injected and filled into the cartilage defect region.

5 Subsequent processes are the same as Example 1.

Meanwhile, when transplanting the fibrinogen matrix formed by adding other mixtures to the fibrinogen, the kinds of the mixtures added to the fibrinogen may vary depending on conditions and age of the patient. For older patients, matrix components such as collagen and hyaluronic acid may be mixed and used with the cartilage therapeutic agent.

10 Fig. 7 shows the differentiation pattern of chondrocytes in the fibrinogen matrix as described above. Differentiation patterns of chondrocytes in the fibrinogen matrix containing 10% collagen and 10% hyaluronic acid are shown in Fig. 8.

### Example 3

15 Injection of a cartilage therapeutic composition using periosteum:

First, cartilage tissue was collected from a patient and chondrocytes were isolated from the tissue and cultured. This was followed by preparation of a cartilage therapeutic agent consisting of more than about  $1.2 \times 10^7$  chondrocytes and 0.4 mL of DMEM.

20 Then, as a commercially available medical product, a fibrin sealant was prepared and DMEM was added to a vial containing lyophilized fibrinogen such that the fibrinogen was dissolved to a concentration of 50 mg/mL to 80 mg/mL.

Additionally, DMEM was added to a vial containing lyophilized thrombin such that the thrombin was dissolved to a concentration of 500 IU/mL.

Then, a site for transplanting a cartilage therapeutic composition was smoothly trimmed using a surgical instrument.

After completion of the above-mentioned process, a periosteum sample was collected from the patient's knee and then was carefully sutured to the transplantation site.

5 After completion of suturing, a sealant was applied to the sutured site.

Then, it was determined whether watertight integrity of the sutured periosteum was complete or not.

Next, to the previously prepared chondrocyte components was added a solution of dissolved thrombin in the concentration of 1 IU/mL to 10 IU/mL, followed by thorough mixing.

10 Next, 0.4 mL of chondrocyte components mixed with thrombin was mixed well with 0.4 mL of a fibrinogen solution to form a transplantation composition. The resulting composition was injected into the periosteum of the affected part using a syringe, as shown in Fig. 5.

In this connection, since fibrinogen reacts with thrombin followed by conversion into fibrin, which in turn clots, injection should be performed rapidly.

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#### Example 4

Injection of a composition made up of a mixture of a fibrinogen matrix in which hyaluronic acid and GAG were added to fibrinogen and cellular components to which thrombin was added:

20 A portion of cartilage tissue was collected from a 27-year old male suffering from cartilage damage due to a traffic accident and cultured to prepare about  $3 \times 10^7$  cells/mL.

As constituents of a fibrinogen matrix, 50 mg/mL to 100 mg/mL of fibrinogen, 10 mg/mL of hyaluronic acid and 5 mg/mL to 10 mg/mL of GAG were prepared.

Since the lesions on the patient due to cartilage damage resulting from the traffic accident

had a size of 4 to 8 cm<sup>2</sup>, 0.5 mL of 50 mg/mL to 100 mg/mL fibrinogen, 0.2 mL of 10 mg/mL hyaluronic acid and 0.2 mL of 5 mg/mL to 10 mg/mL GAG were mixed.

A site for transplanting the cartilage therapeutic agent was cleanly trimmed and two to five holes having a diameter of 3 mm and depth of 5 mm were made, depending on the size of 5 the damaged area.

Then, chondrocyte components mixed with 5 IU/mL of thrombin, and a three-component mixed fibrinogen matrix were prepared.

Subsequently, 500 IU/mL of high concentration thrombin was evenly sprayed onto the transplantation site using a spray tip. Then, 0.9 mL of chondrocyte components and the three-10 component-mixed fibrinogen matrix were mixed.

The composition made up of the mixed chondrocyte component-fibrinogen matrix (hyaluronic acid+GAG) was slowly applied via the holes of the transplantation site.

Again, 500 IU/mL of high concentration thrombin was sprayed onto the transplanted site and left for about 5 min. The transplantation process was finished with completion of hardening.

15 In this connection, since a young patient exhibits good elasticity of cartilage tissues but has rough and uneven cartilage surfaces due to trauma, the use of hyaluronic acid or GAG may alleviate such disadvantages thus promoting easy cartilage regeneration.

As described above, in accordance with the present invention, provided are advantages such as prevention of cartilage therapeutic agent leakage, a simplified surgical process for 20 maintaining a predetermined shape, and the capability of maintaining excellent cartilage generation due to combined transplantation of in vivo matrix components.

Further, provided are effects such as reduction of surgical operation time when periosteum is not sutured, a simplified surgical process due to utilization of an arthroscope, the capability of

5 maintaining excellent cartilage regeneration due to combined transplantation with in vivo matrix components, and no need for knee incision. Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.